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Effects of an increased temperature regime on the population dynamics and species interactions of marine nematodes

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ABSTRACT

Although changes in average environmental conditions can have serious consequences, the main impacts of global climate change on populations and communities may well result from changes in short-term climate variability. Both an increased frequency and intensity of extremes and changing amplitudes of diurnal temperature fluctuations may affect the fitness of species and the interactions between them. Such changing temperature regimes may affect reproductive success, population dynamics, species interactions and community structure. The present study compares the effects of an increased temperature regime with diurnal fluctuations with those of a constant temperature regime on the fitness and population dynamics of free-living marine nematodes and on their interspecific interactions. Microcosm experiments were performed on two congeneric monhysterid nematode species, which co-occur in their natural habitat, under a constant vs. an increased fluctuating temperature regime. The latter affected population dynamics of single species and altered the outcome of the interspecific interaction from a symmetrical to an asymmetrical inhibitory effect of *D. meyli* over *D. oschei*. Changes in the amplitude of diurnal temperature fluctuations as well as in the frequency of extreme temperatures may be very important determinants of the effects of temperature change on species interactions, potentially affecting assemblage structure and ecosystem functioning.

1. Introduction

Climate change is a phenomenon with great environmental impact and rapidly increasing consequences for the life and future of our planet. Sea-surface temperatures are expected to continue increasing globally and major changes are likely to occur in the hydrological and energy cycles (IPCC, 2007; Pachauri et al., 2014). Apart from average temperatures, extremes are also expected to increase in both magnitude and frequency (Easterling, 2000; Pachauri et al., 2014; Thompson et al., 2013), imposing severe stress on a wide range of living organisms.

Along with the predicted episodic temperature extremes, changes in the amplitude of daily fluctuations are also expected in the coming years (Meehl et al., 2000; Walther et al., 2002), rendering climate change effects biologically even more pronounced. In intertidal areas, the combination of episodically elevated water temperature and short-term exposure to high air temperature during low tide may have an especially prominent effect on the development of vulnerable species and on their interactions with other species (Brierley and Kingsford, 2009). In this highly variable environment, temperature regimes may exceed the physiological tolerance limits of particular species, resulting in local extinctions and altering overall ecosystem functioning (Thomas et al., 2004). To date, studies on the effects of fluctuating versus constant temperature regimes in marine invertebrates are scant (De Meester et al., 2015a; Johnson and Shick, 1977; Macheriotou et al., 2015, the latter introducing gradually increasing constant temperature regimes).

Besides individual species fitness, the effects of short-term temperature variation on species interactions can also be significant, though they have hitherto received limited attention (De Meester et al., 2015a; Walther et al., 2002). Effects of climate change on biotic interactions may reflect shifts of dynamics in a community (Gilman et al., 2010) and consequently of ecosystem functioning (Birchenough et al., 2015; Brierley and Kingsford, 2009; Traili et al., 2010). Divergent responses of competing species to climate change may disrupt biological interactions (Poloczanska et al., 2008; Walther et al., 2002). Therefore, any differential response of competitors to thermal stress may either alter or enhance their coexistence (De Meester et al., 2011; De Meester et al., 2015a, 2015b; Descamps-Julien and Gonzalez, 2005). A recent experimental study has, for instance, revealed a shift from commensalism to mutualism for two cryptic species of a marine nematode species complex under constant versus daily fluctuating temperature conditions, respectively (De Meester et al., 2015a).

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Free-living nematodes are excellent model organisms to study responses to thermal stress. They are ubiquitous and highly abundant in marine sediments, they often have a short life cycle and development time, and they are involved in important ecosystem functions, such as nutrient mineralization and organic matter decomposition (Coull, 1999; De Mesel et al., 2006). Additionally, many species are characterized by high phenotypic plasticity and rather wide tolerance limits to disturbance (Bongers and Ferris, 1999; Moens et al., 2013). Some species of the family Monhysteridae, with those of the genus *Diplolaimelloides* Meyl, 1954 often being among the most prominent, are particularly interesting for the study of interspecific interactions, because pronounced competitive or inhibitory interactions exist among them (De Mesel et al., 2006; dos Santos et al., 2009; dos Santos and Moens, 2011), and because they are easily maintained in culture (Moens and Vincx, 1998). Thus, these species offer a favorable model to address hypotheses on the effects of climate change-related thermal stress on horizontal – i.e. within the same trophic level – interactions.

The present study compares the fitness of two marine nematode species under different temperature regimes. Specifically, (a) the response of two congeneric species of the Monhysteridae at the individual and population level, and (b) the outcome of their interactions are investigated under an increased daily fluctuating temperature regime versus a constant temperature regime in a microcosm set-up.

We therefore hypothesized that (a) an increased fluctuating temperature regime would affect population fitness and dynamics, as has been previously observed for *Diplolaimelloides* species under increased constant temperatures (Moens and Vincx, 2000a; Warwick, 1981). Shorter development times and faster population growth rates would be expected under the fluctuating temperature regime because it comprised higher temperatures compared to the constant temperature in our experiment, whereas (b) the response of the two species would be expected to be similar in single-species populations. Given that strong competitive interactions have been reported between the two *Diplolaimelloides* species (De Mesel et al., 2006; dos Santos et al., 2009), we expected (c) that the growth of their populations would be controlled by the strength of their competition and that different temperature regimes may induce shifts in the interactions. Therefore, this work is not only important for predicting climate change effects on biological systems, but also for understanding biotic interactions of closely related species under fluctuating temperature regimes.

2. Materials and methods

2.1. Test organisms

Test organisms for our experiments were two bacterivorous nematode species of the genus *Diplolaimelloides* (family Monhysteridae): *Diplolaimelloides meyli* Timm, 1961 and *D. oschei* Meyl, 1954. The genus *Diplolaimelloides* comprises opportunistic colonizers of decaying organic matter, mostly of vascular plant origin, in coastal and estuarine habitats such as salt marshes and mangroves (Somerfield et al., 1998; Warwick, 1981). *D. meyli* and *D. oschei* are abundant and co-occur in 'Aufwuchs' assemblages of the *Spartina anglica* vegetation zone in salt marshes in the Scheldt Estuary, The Netherlands (Moens, 1999; Moens and Vincx, 2000a). Temperature in their natural habitat can easily exceed 25 °C during low-tide exposure in warm-sunny spring and summer days, consistent with the fairly high upper temperature tolerance limits for the genus *Diplolaimelloides* (Moens and Vincx, 2000a, 2000b; Warwick, 1981).

2.2. Nematode cultures

Nematodes used in the experiments were obtained from monospecific cultures in exponential growth phase, with unidentified bacteria from their natural habitat. *Diplolaimelloides oschei* originated from the Paulina salt marsh in the Scheldt Estuary, The Netherlands, whereas *D. meyli* was originally isolated more upstream (Walsoorden) in the same estuary. Details on the isolation and cultivation of the species are described in Moens and Vincx (1998). Stock cultures of the two species had been maintained in the laboratory under 20 °C temperature and salinity of 25, for many generations prior to the start of our experiments.

2.3. Experimental set-up

Two experiments were conducted simultaneously: one where nematodes of each of the two species were inoculated separately (monospecific experiment: ME) and the other where the two species were inoculated together in the same microcosms (interaction experiment: IE).

The amplitude of the fluctuations was based on climate change scenarios, starting from a close to optimal temperature for the two species (20 °C) (Moens and Vincx, 2000a, 2000b), which is also close to average daily temperatures during summer in the field sites from which both species were isolated. This temperature was also chosen for the constant temperature treatment. Our fluctuating temperature regime increased temperature from 20 °C to 32 °C and back over a daily cycle, and simulated a realistic situation which may easily occur, for instance at the surface of sediments or of cordgrass leaves where the two species abound, in the high intertidal zone of estuarine or marine coastal areas during summer. The maximum temperature was chosen a few degrees below the upper thermal tolerance limit of the test species, which is ca. 35 °C (Moens and Vincx, 2000a and unpubl.).

Experimental microcosms were established in small Petri dishes (5.4 cm int. diam.) filled with 4 ml of 0.75% bacto-agar, prepared with sterile artificial seawater (ASW, Dietrich and Kalle, 1957) with a salinity of 25 and pH 7.5–8. A small amount of cholesterol (100 μl l^{-1}) was added after sterilization of the agar medium as a source of sterols. Frozen-and-thawed *Escherichia coli* (strain K12) were used as food in a concentration of 3×10^9 cells ml^{-1}, which corresponds to the optimal food concentration for both *Diplolaimelloides* species (dos Santos et al., 2008, 2009). 150 μl of the *E. coli* suspension were added on, and spread over the surface of each agar plate at the beginning of the experiment; the same amount was then added as food supply every 10 days until the end of the experiment.

20 active adult nematodes of the same species in ME and of both species together (10 adult individuals of each species) in IE were inoculated in the microcosms according to a replacement design, with a ca. 1:1 sex ratio. The nematodes were randomly collected from the stock cultures using a fine Tungsten wire and transferred to a 10-μl drop of sterile ASW on the agar surface in the middle of each plate.

The experiments lasted 46 days to assess fitness of marine nematodes at the population level under the two different temperature regimes for at least two filial generations, given a generation time of ca. 16 to 18 days for both species (dos Santos et al., 2008; Moens and Vincx, 2000a). In total 256 microcosms were incubated. Four replicate starting populations of single species and of two-species combinations per day of measurement (×16 days of measurement, total 128 microcosms) were incubated under constant temperature at 20 °C (control). The same numbers were incubated under daily fluctuating temperature (treatment). The temperature increased gradually from 20 °C to 32 °C during 10 h and the maximal temperature was maintained for 2 h; then temperature decreased gradually back to 20 °C during 10 h and 20 °C was maintained for 2 h. Four replicate plates per treatment were removed every 48 h for the first 18 days of the experiment and then every 92 h until the end of the experiment (46 days) and were stored frozen at −20 °C. Prior to counting, agar was melted in distilled water (70 °C) and sieved over a 10-μm mesh to retain adults, juveniles and eggs.

Measurements included: total numbers of eggs, juveniles and adult nematodes (males and females) per species for the ME, total numbers of eggs and juveniles of the two species together and number of adult nematodes (males and females) per species for the IE. Counts were

performed using a stereomicroscope. The sex ratio was calculated as the percentage of females in the adult population. For the parental generation (P, i.e. the inoculated adults): (1) total fecundity, calculated as the sum of numbers of eggs and juveniles per female until the onset of reproduction of the first filial generation (F1, i.e. the progeny of the parental generation), (2) daily fecundity, determined as the total fecundity at the time of maturation of the first F1 progeny divided by the number of days required to reach the first F1 adults, and (3) minimum egg deposition time, as the time of the first egg observation, were recorded. In the following results we focus on daily fecundity rather than on total fecundity; estimates of the latter underestimated real fecundity as the parental generation was still reproductively active when we stopped counting progeny. This because progeny could no longer be unambiguously assigned to the parental generation as a result of the onset of reproduction of F1-adults (the reproductive period of P-females lasted up to ca. 22 days, personal observations). Therefore, daily fecundity was a better proxy of reproductive fitness than total fecundity in our experiment. Life-history traits examined only for the first filial generation (F1) are: (4) minimum embryonic development time, calculated by subtracting the minimum egg deposition time from the time of first egg hatching; (5) minimum post-embryonic development time, being the time for the development of freshly hatched juveniles into adults, calculated by subtracting the timing of the first observation of a juvenile from the time of the first observation of a F1-adult; and (6) minimum generation time, recorded as the time interval from the inoculation to the appearance of the first F1-adult (Moens and Vincx, 2000a).

2.4. Data analysis

2.4.1. Monospecific experiment: testing the effect of an increased temperature regime on single-species fitness and population development

The effect of the increased fluctuating temperature regime on single-species fitness was tested by assessing trends in the following life-history traits: minimum time until first egg deposition, embryonic and post-embryonic development time, and minimum generation time, across temperature treatments and species. Since the assumption of normality was not met, even after data transformation, Permutational Analysis of Variance (PERMANOVA) with Bray-Curtis index and 999 permutations was used to test for differences in a factorial design with main factors: temperature and species. Significant effects were further investigated using posterior pair-wise comparisons with a Bonferroni correction. PERMANOVA was conducted using the vegan package in R (Oksanen et al., 2016). The assumptions of normality and homogeneity of variances were tested by Shapiro-Wilk and Levene's tests and by visually inspecting the diagnostic plots (histogram of residuals, Q-Q plots, square root-transformed residuals vs. fitted values and standardized residuals vs. leverage plots). The effect of the increased temperature regime on daily fecundity was tested using two-way Analysis of Variance (ANOVA) in a factorial design with main factors: temperature and species on the $\log(x+1)$-transformed data. All analyses were performed in R (R Core Team, 2016).

The effect of the increased temperature regime on total population development over time was tested by fitting a mixed-effects model to the log-transformed count data. The natural logarithm transformation was applied to total population numbers, i.e. sum of counts of juvenile and adult individuals, to comply with the assumption of homogeneity of variances and to correct large-scale differences. The factors temperature and species were determined as fixed effects, while interaction terms were non-significant and were therefore excluded from the final model (after model validation; nested models were compared by χ^2-test). Replicate sample IDs were introduced to the model as random effects, to account for random biological variability among replicate sampled populations. Temporal continuous autoregressive correlation structure (corCAR1) was introduced to account for the co-variance of the dependent variable in time. The second-degree polynomial regression was preferred over the linear regression to account for the curvilinearity of the data. Normality and homogeneity of model residuals across time were inspected using Q-Q plots and by plotting the residuals vs. the explanatory variables. Different mixed models, with random intercept, with random intercept and slope, and with random effects were compared (Zuur et al., 2009). The mixed models were constructed using the nlme package in R (Pinheiro et al., 2016) and compared using maximum likelihood estimation (ML) and the Akaike Information Criterion (AIC); the random intercept model was finally preferred. Coefficients and *p*-values of the final model were obtained using restricted maximum likelihood estimation (REML).

The effect of the increased temperature regime on adult population development was tested by fitting a similar mixed-effects model to the log-transformed data on adults. For curve fitting purposes, and to allow comparisons with the data of IE (see Section 2.4.2), the N_1 used in the model were those for time $t_1 \geq 14$ days, which corresponds to the t_0 (start) of the exponential growth phase of the curve. Per-generation comparisons were obtained by separately modeling the adult data N_1 for time t_1: $14 \leq t_1 \leq 30$ days for the F1-generation and N_2 for time t_2: $30 \leq t_2 \leq 42$ days for the F2-generation, generations being assigned here by the interval between the onset of reproduction of each progeny generation and the onset of reproduction of its parental generation (see Fig. 3). Average sex ratio data over time were compared across temperature and species using PERMANOVA since assumptions for ANOVA were not met. All analyses were performed in R (R Core Team, 2016).

2.4.2. Interaction experiment: testing the effect of an increased temperature regime on single-species adult population development when species co-occur

The effect of the increased temperature regime on adult population development for species in the IE was tested by fitting a mixed-effects model to the log-transformed data, similar to that fitted on the ME data (see Section 2.4.1), with temperature and species as fixed effects. Non-significant interaction terms were excluded from the final model. For curve fitting purposes and to allow comparisons with the adult data of ME, the adult N_2 used in the model were those for time $t_2 \geq 18$ days, which corresponds to the time of the exponential curve when $N_2 \approx 20$, the starting population of ME. Diagnostic tests, model validation and final model selection were performed in a similar way to what has been described for ME in Section 2.4.1. Average sex ratio data over time and minimum generation times were compared across temperature regimes, species and experiments (i.e. ME vs. IE) in a 3-way factorial design using PERMANOVA and 999 permutations, the assumption of normality not being met.

2.4.3. Interaction experiment: testing the effect of an increased temperature regime on species interaction

To test the effect of the increased fluctuating temperature regime on species interaction, data were analyzed under two scenarios: the real scenario (RS) and a hypothetical scenario (HS). In RS, total numbers of individuals from the interaction experiment were used (total N of the two species together). In HS, data comprised the sum of total individual numbers of the two species from the monospecific experiment (total $N = N_{pop1} + N_{pop2}$ of the mono-populations). When interactions between the two species are insignificant, RS and HS should yield equal results.

The effect of the increased temperature regime on total population development was tested by fitting a mixed-effects model to the log-transformed data for each scenario separately, with temperature as a fixed factor (see Section 2.4.1). Data were first rescaled to allow for comparable model intercepts, as the different starting nematode densities in RS and HS, i.e. 20 and 40 inoculated individuals, respectively, would not allow for a direct comparison of population development under the two scenarios. Thus, the total N_1 used in the model for HS were those for time $t_1 \geq 4$ days, which corresponds to the t_0 (start) of the exponential growth phase of the curve when $N_1 = 40$, the initial starting population for HS. The total N_2 data used in the model for RS were those for $t_2 \geq 6$

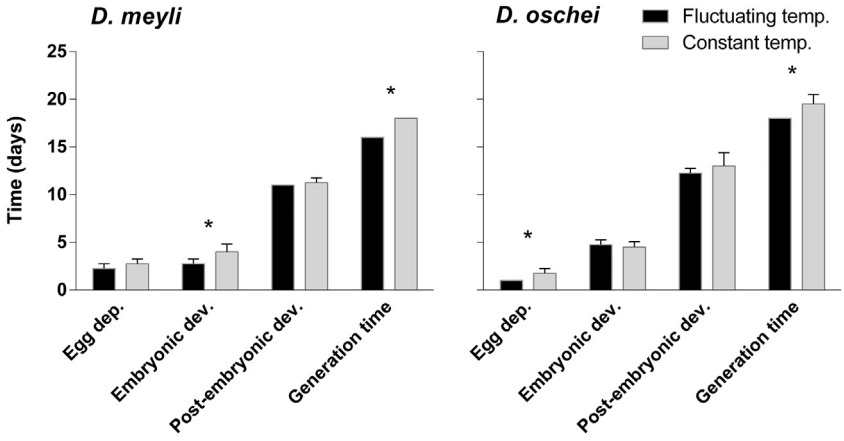

Fig. 1. Life-history features of single-species development in fluctuating and constant temperature conditions in the monospecific experiment. Data are average values ± SD of four replicate populations per treatment; * indicates significant differences between temperature treatments.

days, which corresponds to the onset of the exponential growth phase when the total number of individuals $N_2 \approx 40$, i.e. the same as the starting population of the HS. Diagnostic tests, model validation and final model selection were performed as described in Section 2.4.1. Differences in population development under the two scenarios were observed visually on the fitted growth curves and by comparing the coefficients of the two models. Model parameters were estimated by random sampling with replacement, i.e. non-parametric bootstrapping with 1000 replications, using the boot package in R (Canty and Ripley, 2016; Davison and Hinkley, 1997). The studentized 95% confidence intervals of the bootstrapped parameters of the two models were compared.

A two-way factorial ANOVA was performed on the log(x + 1)-transformed data of daily fecundity of the parental generation (P) to test across scenarios and temperature regimes. Species composition of the adult population in IE (= the real scenario) was modeled over time as a function of temperature regime, the dependent variable being the proportion of adult *D. meyli* over the total number of adult individuals. A mixed-effects model with temperature as the main factor was fitted to the data after log(x + 1) transformation, interaction terms being included. Model diagnostics and selection were similar to what has already been described in Section 2.4.1.

3. Results

3.1. Monospecific experiment: the effect of the increased temperature regime on single-species fitness and population development

3.1.1. Life-history features of single-species development

Overall, shorter development times were obtained in the treatments under fluctuating temperature (Fig. 1). Minimum egg deposition time was temperature and species-dependent, the interaction term also being significant (Table 1). A shorter egg deposition time was observed under fluctuating temperature for *D. oschei* compared to the control (1 vs. 2 days), and to that of *D. meyli* in the control (2.75 ± 0.5 days; $p < 0.05$; Fig. 1).

A significant interaction effect was revealed between temperature and species for the minimum embryonic development time (Table 1), mainly attributed to the shorter embryonic development time of *D. meyli* in fluctuating temperature (2.75 ± 0.5 days) compared to *D. oschei* in fluctuating temperature and control (4.75 ± 0.5 and 4.5 ± 0.58 days; $p = 0.003$ and $p = 0.007$, respectively; Fig. 1). Temperature did not have a significant main effect on the minimum embryonic development time, which did, however, differ between species (Table 1).

Minimum post-embryonic development time also differed significantly between species, with shorter times for *D. meyli* than for *D. oschei*, but did not differ between temperature treatments for both species, the interaction term also being non-significant (Table 1).

Minimum F1-generation time was temperature and species-dependent, the interaction term being non-significant (Table 1). A shorter F1-generation time was observed under fluctuating temperature for both species compared to the control (*D. meyli:* 16 vs. 18 days; *D. oschei:* 18 vs. 19.5 ± 1 days) and for *D. meyli* compared to *D. oschei* (Fig. 1).

3.1.2. Single-species reproductive success

Temperature regime significantly affected the daily fecundity of both species, higher reproductive rates being recorded for the treatments in increased fluctuating temperature regime compared to the control; the interaction term of temperature and species was non-significant (two-way ANOVA: Temp: $F = 14.18$, $p = 0.003$; Sp: $F = 1.29$, $p = 0.278$; Temp × Sp: $F = 0.47$, $p = 0.507$; Fig. 2).

3.1.3. Single-species population development

Increased fluctuating temperature had a significant effect on total population development over time for both species, the population growth rate being significantly higher in treatments under fluctuating compared to constant temperature (Temp: $t = 3.4$, $p = 0.005$; Table 2). The two species responded similarly to the temperature regimes (Sp: $t = -1$, $p = 0.345$; Table 2). Total population abundances of both species increased exponentially until the 38th day of the experiment, then reaching a plateau (Fig. 3). Regardless of the overall increased population growth of treatments at increased fluctuating temperature when compared to the control, the control reached its maximum abundance on day 38 for *D. meyli*; a steep decline was subsequently observed at the constant but not the fluctuating temperature (Fig. 3).

Table 1
PERMANOVA results for the life-history traits of single-species populations. Significant terms are indicated with *.

Measurement	Temperature		Species		Temp × Sp	
Min. time	*Pseudo-F*	*p*	*Pseudo-F*	*p*	*Pseudo-F*	*p*
Egg deposition time	34.11	0.001*	50.00	<0.001*	14.44	0.002*
Embryonic development time	3.37	0.078	15.31	0.002*	5.96	0.023*
Post-embryonic development time	1.45	0.268	14.91	0.004*	0.30	0.515
Generation time	55.48	<0.001*	55.48	<0.001*	2.49	0.141

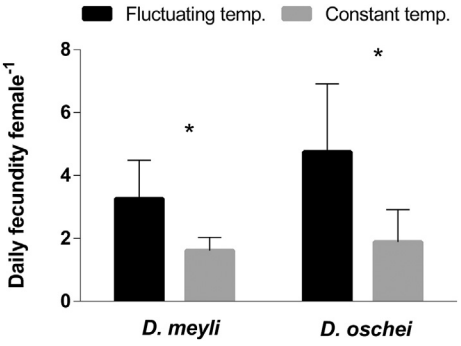

Fig. 2. Daily fecundity of the parental generation per species in fluctuating and constant temperature conditions in the monospecific experiment. Data are average values ± SD of four replicate populations per treatment; * indicates significant differences between temperature treatments.

Adult population development over time was also dependent on temperature, with fluctuating temperature treatments showing faster adult development compared to those under constant temperature ($t = 2.7$, $p = 0.019$; Table 2). There were overall no significant differences between the two species ($t = 1.5$, $p = 0.162$; Table 2). Nevertheless, *D. oschei* reached higher numbers of adults at the F1-generation than *D. meyli* (Fig. 3). This effect, however, changed in the F2 generation, when *D. oschei* reached lower peak numbers of adults than in the F1-generation, whereas the opposite was observed for *D. meyli* (Fig. 3). Per-generation comparisons thus revealed a significant effect of the fluctuating temperature treatment compared to the control for both species in the F1 but not in the F2-generation; in the F2, however, there was a borderline significant difference in peak abundances of adults between the two species (for F1: Temp: $t = 2.2$, $p = 0.045$; Sp: $t = 1.3$, $p = 0.204$; for F2: Temp: $t = 0.7$; $p = 0.474$; Sp: $t = 2.1$; $p = 0.052$, respectively).

The sex ratio of both species was independent of temperature regime, the main effect of temperature and the interaction of temperature and species being non-significant (PERMANOVA: Temp: $F = 0.00$, $p = 0.993$; Sp: $F = 8.35$, $p = 0.003$; Temp × Sp: $F = 0.03$, $p = 0.911$). A significant species effect was attributed to the increase of percentage females of *D. oschei* with time (reaching mean ± SD: 56.75 ± 7.81% at the end of the experiment; overall mean ± SD: 50.32 ± 4.11%), whereas no significant change was observed for *D. meyli* (48.88 ± 3.52% at the end of the experiment; overall mean: 48.84 ± 4.05%; Fig. 4).

Table 2
Mixed-model parameters and statistics for the total population (Total N) and the adult population (Adult N) abundances in the mono-species experiment (model fit by Restricted Maximum Likelihood method, REML). Significant terms are indicated with *.

Total N					
Main effects	**Value**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	5.66	0.06	253	98.4	<0.001
Time	32.90	0.52	253	63.0	<0.001*
Time^2	−3.97	0.51	253	−7.9	<0.001*
Temperature	0.22	0.06	13	3.4	0.005*
Species	−0.06	0.06	13	−1.0	0.345
Random effects	**(Intercept)**	**Residual**	**AIC**	**BIC**	**logLik**
SD	1.54 10^{-5}	0.45	339	368	−162
Adult N					
Main effects	**Value**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	4.08	0.08	142	51.1	<0.001
Time	6.85	0.58	142	11.9	<0.001*
Time^2	−4.42	0.54	142	−8.2	<0.001*
Temperature	0.25	0.09	13	2.7	0.019*
Species	0.14	0.09	13	1.5	0.162
Random effects	**(Intercept)**	**Residual**	**AIC**	**BIC**	**logLik**
SD	2.65 10^{-5}	0.49	241	266	−113

3.2. Interaction experiment: the effect of the increased temperature regime on adult population development of both species in interaction treatments

The adult population of the two species in interaction followed a similar logarithmic growth curve (Sp: $t = -0.7$, $p = 0.507$; Table 3), with faster development rate in the early days which then slowly declines with time (Fig. 5). Higher numbers of adults were observed for *D. meyli* compared to *D. oschei* under increased fluctuating temperature, whereas *D. oschei* reached higher numbers in control, those differences being more pronounced towards the end of the experiment (significant interaction term of Temp × Time^2: $t = 2.5$, $p = 0.016$; Table 3; Fig. 5). However, fluctuating temperature did not show a significant main effect on adult population development for either species in interaction (Temp: $t = -0.2$, $p = 0.838$; Table 3).

Sex ratio did not differ significantly across experiments (ME vs. IE) or temperature treatments (PERMANOVA: Ex: $F = 0.77$, $p = 0.388$; Temp: $F = 1.49$, $p = 0.215$, all interaction terms: $p > 0.05$). A difference was observed between species ($F = 21.18$, $p = 0.001$) with the sex ratio of *D. oschei* not changing significantly over time in IE (*D. oschei*: mean ± SD: 50.63 ± 10.56% at the end of the experiment; overall mean ± SD: 50.63 ± 6.59%), whereas a small decline was observed for *D. meyli* (46.38 ± 6.30% at the end of the experiment; overall mean: 49.62 ± 6.44%; Fig. 6).

Minimum F1-generation time was temperature and species-dependent. The interaction terms of temperature and experiment, and of species and experiment were also significant (PERMANOVA: Temp: $F = 55.48$, $p < 0.001$; Sp: $F = 55.48$, $p < 0.001$; Ex: $F = 2.49$, $p = 0.131$; Temp × Sp: $F = 2.49$, $p = 0.141$; Temp × Ex: $F = 55.48$, $p < 0.001$; Sp × Ex: $F = 55.48$, $p < 0.001$; Temp × Sp × Ex: $F = 2.49$, $p = 0.129$). Shorter generation times of *D. meyli* compared to *D. oschei* and in fluctuating temperature treatments compared to control were observed in ME ($p < 0.05$; Fig. 1), whereas the minimum generation time for all treatments in IE was 18 days.

3.3. Interaction experiment: the effect of the increased temperature regime on species interaction

3.3.1. Reproductive success of species in interaction

Temperature regime did not have a significant effect on daily fecundity of the parental generation (P) for the two-species population, for either scenario, although fecundity rates in fluctuating temperature treatments were generally higher compared to constant temperature in both scenarios (two-way ANOVA: Temp: $F = 2.93$, $p = 0.113$; Scenario: $F = 3.32$, $p = 0.094$; Temp × Scenario: $F = 0.17$, $p = 0.685$; Fig. 7).

3.3.2. Population development of species in interaction

Increased fluctuating temperature had a significant main effect on total population development over time for the HS but not for the RS, the population growth for the HS being higher in treatments with fluctuating temperature compared to the control (Temp effect for HS: $t = 2.9$, $p = 0.026$; Temp effect for RS: $t = 0.1$, $p = 0.954$, respectively; Table 4). Comparable to what has been reported for the mono-populations in ME (Section 3.1.3), total population numbers in HS increased exponentially until reaching their carrying capacity; a steep decline was observed on the 38th day for the population of the control (Fig. 8). The biological interaction showed a significant effect on the total population development, with the exponential growth for the RS being smoother compared to that in the HS and not reaching a plateau during the course of the experiment (Table 4; Fig. 8; the bootstrapped Time coefficients predicted by the two models significantly differed (RS: 12.65 ± 0.90; HS: 20.30 ± 0.88; Studentized 95% CI: RS: (6.109, 6.682), HS: (6.316, 7.047); see ESM 1).

Increased fluctuating temperature had a significant effect on species composition over time for the real scenario (RS), the main effect also being significant (Temp: $t = 2.7$, $p = 0.034$; Temp × Time: $t = 3.3$, $p = 0.001$; Table 5). The proportion of adult *D. meyli* over the total

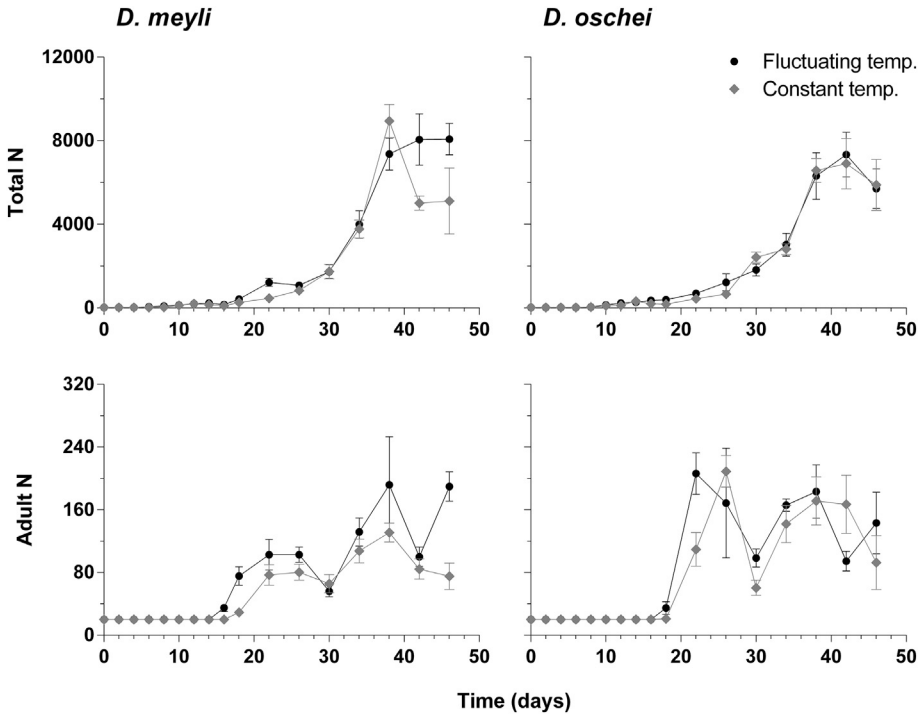

Fig. 3. Total population (Total N) and adult population (Adult N) abundances per species as a function of time under fluctuating and constant temperature conditions in the monospecific experiment. Data are average numbers of individuals ± SE of four replicate populations per treatment.

number of adult individuals increased over time from the initial 0.5 (equal numbers of both species at the start of the experiment) to 0.64 ± 0.07 (mean ± SD) at the end of the experiment, in treatments under fluctuating temperature regime. Species composition in treatments under constant temperature, though, did not change significantly over time (0.46 ± 0.11 at the end of the experiment; fixed time effects: $p > 0.05$; Table 5; Fig. 9).

4. Discussion

The present study reveals that increasing temperatures of a daily fluctuating regime affect population dynamics and fitness of single species. By contrast, that effect is not significant when the two species co-occur, the interaction between the two species being the leading factor influencing species-specific population dynamics. Nevertheless, shifts in species composition related to temperature regimes may alter the species relationship or the direction of the interspecific interaction and, consequently, alter community dynamics.

4.1. The effect of the increased temperature regime on single-species fitness and population dynamics

An overall significant effect of the increased fluctuating temperature regime on the fitness of the two nematode species in monoculture was revealed. The faster population development under daily fluctuating temperature compared to the constant temperature regime can be attributed to the effect of the higher mean temperature of that regime instead of the fluctuations per se. The logistic type of the total population growth curves indicates that the two populations are regulated by density dependence and resource limitation (Gurney and Nisbet, 1998; Schoener, 1973). The two populations seem to have reached their very similar carrying capacity on the 42nd day of the experiment, with the exception of the *D. meyli* population at constant temperature which

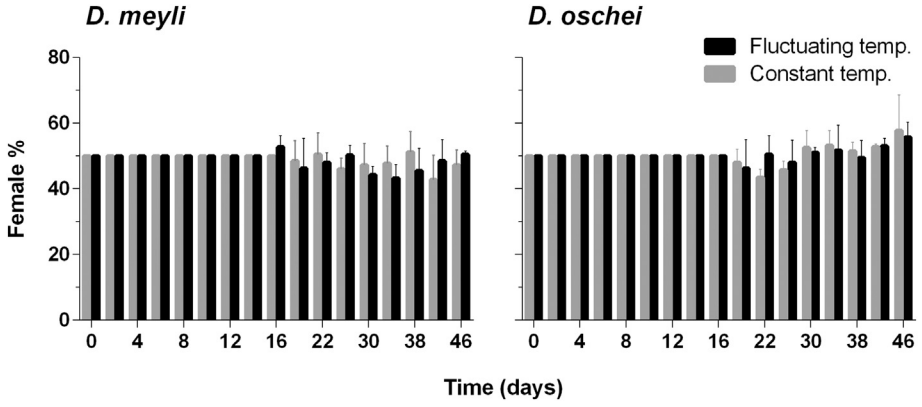

Fig. 4. Sex ratio (as female percentage) of the adult population per species in time under fluctuating and constant temperature conditions in the monospecific experiment. Data are average numbers of individuals ± SD of four replicate populations per treatment.

Table 3
Mixed-effects model parameters and statistics for the adult population abundance (Adult N) of the interaction experiment (model fit by Restricted Maximum Likelihood method, REML). Significant terms are indicated with *.

Total N					
Main effects	**Value**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	3.46	0.06	108	55.7	<0.001
Time	3.77	0.50	108	7.5	<0.001*
Time^2	−1.51	0.48	108	−3.1	0.002*
Temperature	−0.02	0.07	13	−0.2	0.838
Species	−1.04	0.07	13	−0.7	0.507
Time × Temp	−1.27	0.07	108	−1.8	0.076
Time^2 × Temp	1.67	0.68	108	2.5	0.016*
Random effects	**(Intercept)**	**Residual**	**AIC**	**BIC**	**logLik**
SD	0.06	0.33	101	129	−40.5

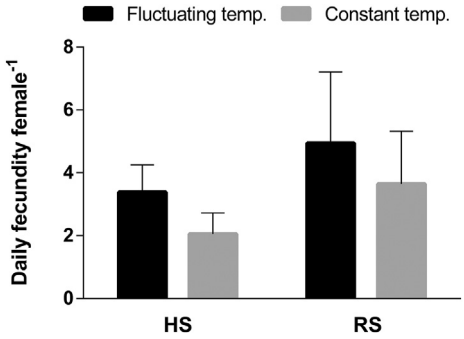

Fig. 7. Daily fecundity of the parental generation of the common population for the hypothesis (HS) and the real scenario (RS) in fluctuating and constant temperature conditions. Data are average values ± SD of four replicate populations per treatment.

suddenly declined from the 38th day onwards. Not only density but also food limitation might have caused the population decline, since the last food replenishment was 8 days before (on the 30th day).

The two species had a similar population development and reached comparable population sizes under constant temperature regime, in agreement with previous experiments under constant temperatures (dos Santos et al., 2008; Moens and Vincx, 2000a). Nevertheless, higher densities of *D. oschei* than *D. meyli* were reported by dos Santos et al. (2008) in monospecific cultures with the same food availability as used in the present study (3×10^9 cells ml^{-1} *E. coli*). The temperature used in that experiment, though, was lower than in our constant temperature regime (17 °C instead of 20 °C), which may suggest that *D. oschei* performs better than *D. meyli* under lower temperature. Another experimental study with *D. meyli* at constant temperatures demonstrated that the maximal population densities reached were largely independent of temperature in the interval from 15 °C to 30 °C (Moens and Vincx, 2000a). Mortality was limited even at 30 °C. Thus, the high total numbers for both single species in the present experiment and their similar response to fluctuating temperature regimes demonstrate the ability of both species to cope with both increasing and fluctuating temperatures. Additionally, in the present study, *D. oschei* reached higher abundances of adults than *D. meyli* in both temperature regimes. This higher number of adults in the first filial generation (F1) is explained by the higher daily fecundity of the parental generation of *D. oschei*, which might in turn have caused the decline in fecundity in the

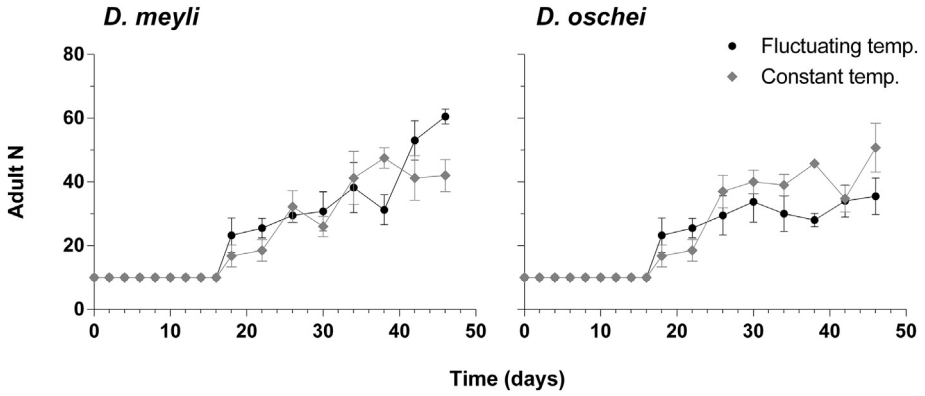

Fig. 5. Adult population development per species in fluctuating and constant temperature conditions in the interaction experiment. Data are average numbers of individuals ± SE of four replicate populations per treatment.

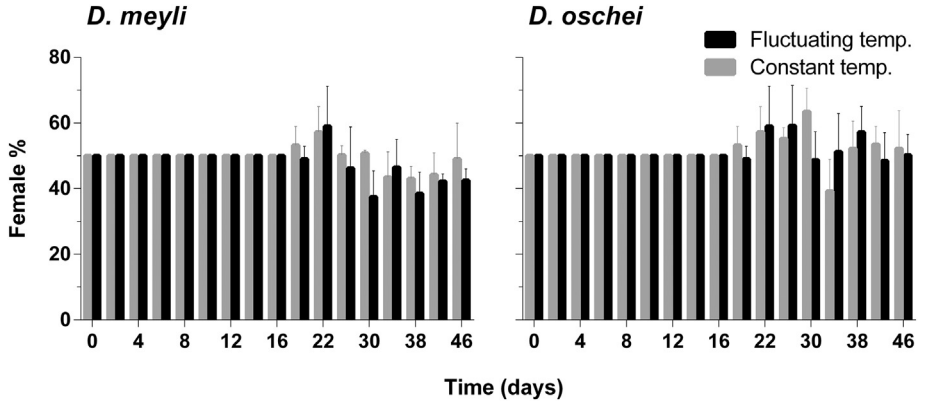

Fig. 6. Sex ratio (as female percentage) of the adult population per species in time under fluctuating and constant temperature conditions in the interaction experiment. Data are average numbers of individuals ± SD of four replicate populations per treatment.

Table 4
Mixed-effects model parameters and statistics for the total population abundances for the Real (RS) and the Hypothesis Scenario (HS) (model fit by Restricted Maximum Likelihood method, REML). Significant terms are indicated with *.

Real Scenario (RS)					
Main effects	**Value**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	6.38	0.08	102	82.6	<0.001
Time	12.89	0.57	102	22.5	<0.001*
Time^2	−3.10	0.56	102	−5.6	<0.001*
Temperature	0.01	0.11	6	0.1	0.954
Random effects	**(Intercept)**	**Residual**	**AIC**	**BIC**	**logLik**
SD	1.34 10^{-5}	0.53	191	210	−88.7
Hypothesis Scenario (HS)					
Main effects	**Value**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	6.68	0.06	109	113.6	<0.001
Time	20.40	0.43	109	47.3	<0.001*
Time^2	−3.36	0.40	109	−8.4	<0.001*
Temperature	0.24	0.08	6	2.9	0.026*
Random effects	**(Intercept)**	**Residual**	**AIC**	**BIC**	**logLik**
SD	8.27 10^{-6}	0.35	95.8	115	−40.9

Table 5
Mixed-effects model parameters and statistics for the species composition of the interaction experiment as proportion of *D. meyli* over the total number of adults (model fit by Restricted Maximum Likelihood method, REML). Significant terms are indicated with *.

Proportion of *D. meyli*					
Main effects	**Value**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	3.40	0.01	100	72.2	<0.001
Time	−0.05	0.06	100	−0.8	0.405
Time^2	0.06	0.06	100	1.0	0.331
Temperature	0.02	0.01	6	2.7	0.034*
Time × Temp	0.27	0.08	100	3.3	0.001*
Time^2 × Temp	0.10	0.08	100	1.2	0.245
Random effects	**(Intercept)**	**Residual**	**AIC**	**BIC**	**logLik**
SD	3.89 10^{-9}	0.04	−351.9	−328	185

second filial generation (F2) due to density dependence of the population. Thus, over the whole duration of the experiment, opposing differences in fecundity between species in the first and second generation canceled each other out, hence the absence of a significant difference in nematode abundances between species.

We expected faster development and hence shorter egg deposition, embryonic and postembryonic development times and generation times at the fluctuating temperature regime because of the higher overall temperature (Heip and Vincx, 1985; Vranken et al., 1988; Warwick, 1981). Minimum egg deposition time was indeed shorter under fluctuating temperature for *D. oschei* but not for *D. meyli*, yet was not followed by a significantly shorter embryonic or post-embryonic development time. The shorter generation times under fluctuating temperature for both species compared to the control are in agreement with similar records at constant temperatures (Moens and Vincx, 2000a), and thus may be explained by an effect of the increased temperatures of the fluctuating regime rather than of the fluctuations per se. Nevertheless, faster reproduction and development of another monhysterid nematode, *Monhystera parva* (Bastian, 1865), under a fluctuating temperature compared to a constant temperature regime with an equal mean temperature and number of degree days have been observed (Moens, unpubl.). Hence, an effect of the temperature fluctuation itself cannot be completely discarded, even though such effect was insignificant in experiments with the species complex of *Litoditis marina* (Bastian, 1865) Sudhaus, 2011 (De Meester et al., 2015a). Reproductive success in our study was also dependent on temperature regime, with higher fecundity under the fluctuating temperature. Increasing reproduction rates with increasing temperatures have been reported in several studies for marine and brackish-water nematodes, fecundity increasing almost until the upper temperature tolerance limit of the species (Heip and Vincx, 1985; Moens and Vincx, 2000a; Vranken et al., 1988; Warwick, 1981).

Sex ratio was independent of temperature regime, following the 1:1 female:male ratio previously reported for *D. meyli* at temperatures >15 °C (Moens and Vincx, 2000a). Hitherto, substantial differences in sex ratio of *D. meyli* in lab experiments at 25 °C have been reported, with the percentage of females ranging from ≤35% (Moens et al., 1996) to 56.5% (Moens and Vincx, 2000a) at 25 °C constant temperature. The difference between those two studies may have resulted from the fact that the former reported on a mixed-species experiment in which *D. meyli* was inoculated together with *Litoditis marina*, whereas the latter was a monospecific experiment. Nevertheless, in the present study, sex ratios did not differ between monospecific and interaction experiment, and only slight deviations from a 1:1 ratio were observed.

Several life-history features observed here were species-specific. Species differences were in agreement with dos Santos et al. (2008), with faster embryonic development and generation times for *D. meyli* compared to *D. oschei*. Such differences could potentially be linked to the previously observed asymmetric competition between these two species, where *D. meyli* was found to be a superior competitor compared to *D. oschei* (De Mesel et al., 2006; dos Santos et al., 2009; dos Santos and Moens, 2011). Our current study, however, does not support this (see further in Section 4.2).

4.2. The effect of temperature regime on interspecific interactions

The relationship between the two *Diplolaimelloides* species has been described as mutual inhibition (De Mesel et al., 2006; dos Santos et al.,

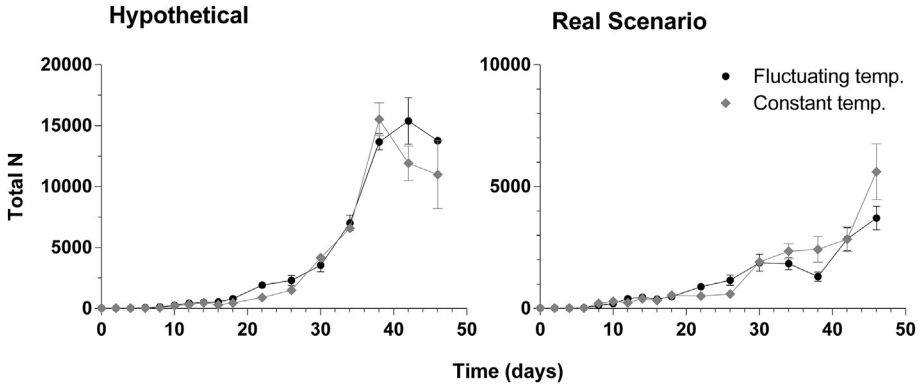

Fig. 8. Total population development for the two scenarios in fluctuating and constant temperature conditions. Data are average numbers of individuals ± SE of four replicate populations per treatment. Note the different Y-axis scale for the two panels.

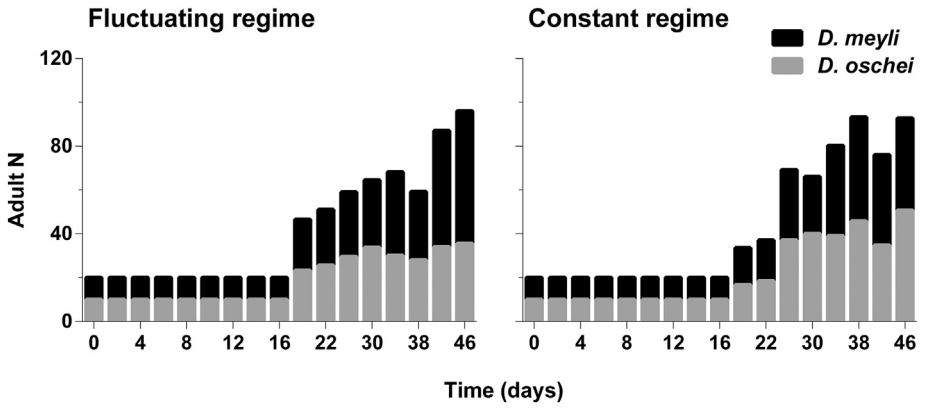

Fig. 9. Species composition of the adult population through time in fluctuating and constant temperature conditions in the interaction experiment. Data are average numbers of individuals of four replicate populations per treatment.

2009; dos Santos and Moens, 2011), with *D. meyli* being a stronger competitor than *D. oschei* under constant temperature. Lower adult population growth rates and lower average population sizes were indeed observed for both species in the interaction experiment compared with single species' adult populations. Studies on the competition of nematode species suggest that the driving factor may be (a) resource depletion (Ilieva-Makulec, 2001), (b) chemical interference (De Mesel et al., 2006) or (c) interspecific copulation resulting in unsuccessful reproduction (dos Santos et al., 2009). The first theory was rejected by De Mesel et al. (2006) and may also not apply in our experiment, apart from the very last days (42nd and 46th day), since the last food replenishment was on the 40th day when population densities were already high. While statistically not significant, daily fecundity in our study tended to be higher in the interaction experiment compared to the combined mono-populations (real vs. hypothesis scenario). Thus, competition had a moderately positive effect on the reproductive success of the two interacting species in both temperature regimes, rejecting the third hypothesis but leaving ground for the idea of allelopathic interactions. This is in line with the observation that juvenile development was reduced by the species interaction, since lower numbers of nematodes of both species matured to adults in the interaction experiment.

Correspondingly, the total population size and the growth rate of the combined population in the real scenario were smaller than of the hypothetical combined population, which was affected by the increased fluctuating temperature regime. Thus, the real combined population did not reach its carrying capacity until the end of the experiment because of the slower population growth rate, limited by competition effects. While these results side with previous studies having reported an inhibitory effect for both species populations (De Mesel et al., 2006; dos Santos et al., 2009; dos Santos and Moens, 2011), they differ in the fact that under the constant temperature regime in our experiment, the interaction was symmetric instead of asymmetric. More specifically, the two species were equally represented under constant temperature, whereas *D. meyli* increased in density towards the end of the experiment under the fluctuating temperature regime, at the expense of *D. oschei*. The latter result agrees with the asymmetric inhibitory effects reported in the above-mentioned studies. This change of direction of the interspecific interaction was revealed as an effect of the imposed fluctuating temperature regime. The population growth of both species decreases because of the interaction effect, but might also meet an equilibrium when both species are equally abundant (species ratio 1:1), which in our experiment was the case under constant temperature (Fig. 10). Under fluctuating temperature, the equilibrium is shifted with *D. meyli* being a stronger competitor than *D. oschei* (Fig. 10). Nevertheless, this equilibrium is not stable, and given the environmental conditions and any change that may occur, especially in the longer-term, the dynamics of the interspecific interaction may easily change.

Regardless of the direction of the interaction, the effect of fluctuating temperature regime on the outcome of interspecific interactions is in agreement with the results by De Meester et al. (2015a) for cryptic species of another marine bacterivore nematode, *Litoditis marina*. Their study also revealed a change from commensalism to mutualism under constant vs. daily fluctuating temperature, respectively. In addition to different temperature regimes, differences in other environmental factors, like salinity, may also affect the nature and/or direction of species interactions (De Meester et al., 2011, 2015b). Hence, such changes in the abiotic-natural environment of the species may induce shifts in

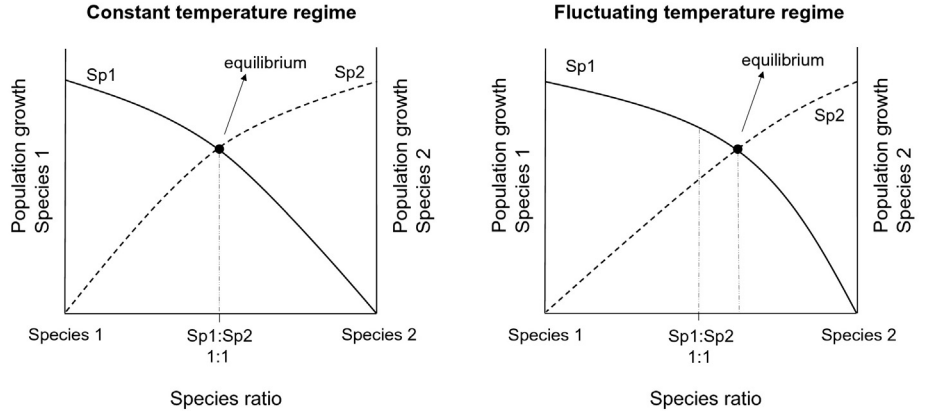

Fig. 10. Schematic representation of the shift in equilibrium of population growth for the two species in interaction in constant and in fluctuating temperature regimes. Species 1 here represents *D. oschei* and species 2 *D. meyli*.

their interactions, which may even translate into changes in ecosystem functioning. Therefore, more complex, indirect effects of climate change should be considered for an accurate forecast of the long-term effects on species relationships (Poloczanska et al., 2008).

5. Conclusion

The results of the present study mainly support the idea that diurnal temperature fluctuations affect single-species fitness and population development and modify their interspecific interaction. Although, species interaction is the main factor shaping population dynamics under different temperature regimes, the increased fluctuating temperature regime may be responsible for shifts in the nature/outcome of that interaction. Since closely related nematode species may respond differently to stress and since they may not have identical functional roles in the ecosystem, shifts in their interspecific interactions may even cause change in ecosystem functioning. Hence, it is more important to focus on, and understand, biotic interactions of closely related species rather than single-species performance. Determining species plasticity and the way interactions among prominent species in a community may alter under thermal stress is necessary for accurate predictions of changes in community dynamics caused by climate change.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2017.02.008.

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